EVALUATION OF MINERALS, ANTI-NUTRIENTS AND MICROBIOLOGICAL ATTRIBUTES OF BREAD PRODUCED FROM WHEAT (*Triticum spp*.), ACHA (*Digitaria exillis*), FLUTED PUMPKIN (*Telfairia occidentalis*) AND FISH (*Clarias gariepinus*) BLENDS

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ABSTRACT
Bread is the staple food eaten in most countries around the world. Evidence has shown that various research teams have worked on fortifying bread due to the demands for healthier food. The aim of this work was to evaluate the minerals, anti-nutrient and microbiological attributes of bread produced from wheat, Acha, fish and pumpkin flour blends. Four composite flour blends were formulated in the ratio of 86:11:0:3, 77:12:8:3, 70:17:10:3, 100:0:0:0 (wheat, acha, fluted pumpkin and fish). The flour blends were used to produce breads using the straight dough method of AOCC. The samples were analyzed according to standard methods. The data were subjected to analysis of variance at 5% level of significance using SPSS. Results showed that minerals were present in appreciable amount in the bread samples calcium (25.99-80.5mg/100g), phosphorous (35.92-58.97mg/100), sodium (10.45-54.88mg/100g), potassium (85.00-115.94mg/100g), iron (10.31-22.43mg/100g) and zinc (1.64-8.07mg/100g). The molar ratio of sodium/potassium and calcium/phosphorous ranged from 0.12 to 0.47 and 0.72 to 1.43, respectively. The molar ratios were lower than critical levels. The anti-nutritional factors: phytate (0.31-0.90mg/g), saponin (0.63-1.26mg/g) and tannin (1.01-3.77mg/g) contents of the bread samples showed no significant ($p < 0:05$) difference except for oxalate (6.12-8.16mg/g) where there was significant difference between samples B and D. Bacteria *E. coli* and Salmonella spp were not detected in all of the bread samples produced but fungi (yeast and mould) were isolated in all the samples. It could be concluded that the incorporation of pumpkin and fish to bread enhanced the nutritional values of the bread.
Keywords: Composite flours, nutritional values, diabetes mellitus, microbial load.

INTRODUCTION
Fortification of food may be used as a tool to correct or prevent widespread nutrient inadequacies and, hence, correct associated micronutrient deficiencies or to restore nutrients lost in food processing, or to make products more appealing to consumers (Dwyer *et al.*, 2015).
Bread is the staple food eaten in countries around the world. The bread produced principally from wheat flour, is rich in carbohydrate and provides more than 50% of the total energy intake. Due to its relatively low cost, availability, acceptability, and widespread consumption, bread is an excellent product in which the incorporation of functional ingredients,such as omega-3 fatty acids, is attempted. Researchers have worked on fortifying bread with natural compounds due to the demands for healthier food (Melilli *et al.*, 2019).
Fluted Pumpkin *(Telfairia occidentalis)* is a tropical Vine grown in West Africa as a leafy vegetable and for its edible seeds. The seeds are cooked and eaten like beans and the shoots and leaves are eaten like vegetables. The nutritional value of fluted pumpkin leaves shows it contains; folate, calcium, copper, niacin, magnesium, potassium, manganese, vitamins A, vitamin. B2, B_6, C_2, and E, flavonoids, and carotene.
Fonio (Acha), is included in a list of grains considered as whole grains when consumed in whole form (Melilli *et al.*, 2019). There is evidence showing that whole grain diets compared with refined grain diets are beneficial for several health outcomes. Thus, this study examined the minerals, anti-nutrient and microbiological attributes of bread produced from wheat, Acha, fish and pumpkin leaf to gain insight on how bread can be fortified to increase the nutritional content with a low glycemic index.

MATERIALS AND METHOD
Pumpkin leaf (*Telfairia occidentalis*), Acha (*Digitaria exilis*), fish (*Clarias gariepinus)* and wheat (T*riticum aestivum)* were purchased from a community market within Lafia Metropolis. Four composite flour blends were formulated in the ratio of 86:11:0:3, 77:12:8:3, 70:17:10:3, 100:0:0:0

(wheat, acha, futedpumpkin and fish.). The flour blends were used to produce breads using the straight dough method of AOAC (2007)

Determination of anti nutrients

Oxalate content of the bread samples was determined using the method of Day and Underwood (1986). Phytate was determined as described by Aemiro *et al* (2020) while saponin and Tannin contents were determined as described by Odebiyi and Sofowora (1978) and Sabris *et al*., (2012) respectively.

Microbial Analysis of Bread

Total mold, yeast, and bacteria counts were carried out on bread samples after 2-day room temperature storage using the method of AOAC (2007).

Determination of Minerals

(P, Mg, K, Na Ca, Mn, Pb, Cu and Fe concentrations were determined using Atomic Absorption Spectrophotometer (Hitachi,model 180-80). Phosphorus was determined Spectrophotometrically by the vanadomolybdate method (AOAC, 2007)**.**

Statistical Analysis

All measurements were done in at least two replicates, and the data were subject to analysis of variance (ANOVA), applying software package SPSS 16.0.The significance of differences was evaluated by Duncan's test, at $P<0.05$.

RESULTS AND DISCUSSION

The result for minerals and vitamins composition of bread samples is presented in Table 1. Results showed that minerals were present in appreciable amount in the bread samples calcium (25.99-80.50 mg/100g), phosphorous (35.92- 58.97 mg/100), sodium (10.45-54.88mg/100g), potassium (85.00-115.94 mg/100g), iron (10.31-22.43 mg/100g) and zinc (1.64-8.07mg/100g). The molar ratio of sodium/potassium (Na/K) and calcium/phosphorous (Ca/P) ranged from 0.12 to 0.47 and 0.72 to 1.43, respectively. Significant difference (P< 0:05) for lead (pb) was observed among all bread samples but was lower than the values recorded for bread consumed in Nigeria by Adebiyi *et al., (*2021), this is a mark of safety as lead exposure has been linked to several neurological and metabolic challenges. Ayo *et al.,* (2018) reported lesser amount of potassium, calcium, phosphorous and sodium for bread made from a blend of Acha and mushroom. The increase in potassium, calcium, phosphorous and sodium in the current study may be attributed to fish and pumpkin supplements. Calcium intake in diabetics has been shown to be beneficial and likely to reduce osteoporosis in older diabetics. The low sodium level observed in this study indicate that the bread would be good for consumption for both diabetes and non-diabetes.

The anti-nutrient composition of bread formulated from wheat flour, Acha, pumpkin and fish is presented in (Table 2). The phytate content ranged from is 0.31 -0.90 mg/g, saponin 0.63- 1.26 mg/g, oxalate 6.12- 8.16 mg/g and Tannin 1.01- 3.77mg/g. The anti-nutritional (phytate, saponin and tannin) content of the bread showed no significant ($p < 0:05$) difference among samples except for oxalate where there was significant difference between sample B(77% Wheat, 12% Acha, 8% fluted Pumpkin leaf, 3% Fish) and D (100% Wheat, 0% Acha, 0% fluted Pumpkin leaf, 0% Fish) respectively. In general, there was increase in anti-nutritional content in bread with high wheat content. The oxalate and tannin were lower than that reported by Karishma and Pravin, (2016) for bread fortified with germinated horse gram (*Dolichus uniflorus*) flour. Ojinnaka and Agubolum (2013) reported higher amount of tannin and phytate in cookies prepared from cashew nut.

Table 1: Mineral composition (mg/100 g)

Parameters	A	B	C	D	*Ref
Ca	25.99 ± 0.05^{d}	46.93 ± 0.10^{c}	80.50 ± 0.14^{a}	57.29 ± 0.09^{b}	500
Mg	2.81 ± 0.02^{d}	5.46 ± 0.03^{b}	7.53 ± 0.02^{a}	4.05 ± 0.03^{c}	76
P	35.92 ± 0.07^{d}	47.87 ± 0.16^{b}	58.97 ± 0.21^{a}	40.30 ± 0.10^{c}	456
K	85.00 ± 0.06^{c}	105.16 ± 0.18^{b}	115.94 ± 0.13^{a}	63.19 ± 0.01^{d}	516
Na	10.45 ± 0.03^{d}	46.93 ± 0.04^{b}	54.88 ± 0.09^{a}	14.72 ± 0.02^{c}	296
Fe	10.31 ± 0.00^{d}	16.29 ± 0.03^{b}	22.43 ± 0.06^{a}	11.06 ± 0.02^{c}	16

Zn	1.64±0.01[d]	2.81±0.03[c]	4.07±0.02[b]	8.07±0.03[a]	3.20
Cu	0.09±0.01[d]	0.53±0.02[b]	0.71±0.02[a]	0.16±0.02[c]	0.89
Mn	0.39±0.02[c]	0.69±0.02[b]	0.83±0.01[a]	0.06±0.01[d]	1.50
Pb	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.02±0.00[a]	0.01
Na/K	0.12[d]	0.45[b]	0.47[a]	0.23[c]	<1.00
Ca/P	0.72[d]	0.98[c]	1.37[b]	1.43[a]	>1.00

Means (±SEM) with different alphabetical superscripts in the same row are significantly different at P<0.05. A = 86% Wheat, 11% Acha, 0% fluted Pumpkin leaf, 3% Fish, B = 77% Wheat, 12% Acha, 8% fluted Pumpkin leaf, 3% Fish, C= 70% Wheat, 17% Acha, 10% fluted Pumpkin leaf, 3% Fish, D = 100% Wheat, 0% Acha, 0% fluted Pumpkin leaf, 0% Fish

Phytate, oxalate, and tannin not only interferes with mineral absorption, but also impair protein digestibility. Malomo *et al.* (2011) reported that 80 mg/g of these anti-nutritional factors are detrimental to health, so the values obtained in this study are considerable lower and therefore safe for consumption.

Table 2: Anti-nutrient composition (mg/g) of bread made from wheat flour, Acha, pumpkin and fish blend.

Parameters (mg/100)	A	B	C	D	*CV
Phytate	0.90±0.03[a]	0.53±0.03[c]	0.31±0.02[d]	0.62±0.03[b]	5-6
Saponin	1.26±0.03[a]	0.82±0.03[b]	0.63±0.03[d]	0.79±0.03[c]	0.25
Oxalate	6.12±0.04[c]	8.16±0.04[a]	7.00±0.01[b]	8.12±0.06[a]	2.5
Tanning	3.77±0.02[a]	1.65±0.02[b]	1.01±0.01[d]	1.34±0.02[c]	3.0

Means (±SEM) with different alphabetical superscripts in the same row are significantly different at P<0.05. A = 86% Wheat, 11% Acha, 0% fluted Pumpkin leaf, 3% Fish, B = 77% Wheat, 12% Acha, 8% fluted Pumpkin leaf, 3% Fish, C= 70% Wheat, 17% Acha, 10% fluted Pumpkin leaf, 3% Fish, D = 100% Wheat, 0% Acha, 0% fluted Pumpkin leaf, 0% Fish

The microbial load of bread is shown in Table 3. Total plate of the bread was highest for Sample A with 3.0 cfu/g for sample A, followed by 2.0 cfu/g for Sample D and 1.0 cfu/g apiece for sample B and C. The bacteria, E. *Coli* and *Salmonella* were absent in all bread samples while yeast and mould recorded 2.0 sfu/g x10^3 for sample B, C and D with the highest (2.0 sfu/g x10^3) for bread made from 86% Wheat, 11% Acha, 0% fluted Pumpkin leaf, 3% Fish. E. *coli* and *Salmonella* were not detected in all of the bread samples produced while fungi (yeast and mould) were isolated in all samples. These however, are within the limit set by the Standard Organization of Nigeria, which states that the counts of aerobic bacteria must not exceed 100 cfu/g and coliform growth must not be detected in bread samples while total plate count values for cereal and legume-based products exceeding 106cfu/g are considered microbiologically unsafe (Daniyan and Nwokwu, 2011). From this study, none of the treatment exceeded 106 cfu/g. This shows that such bread is safe for consumption as there is no fecal contamination. The fungus population could be due to the introduction of yeast as ingredient for baking. The fungi (yeast and mould) counts were higher in bread made from sample A (86% Wheat, 11% Acha, 0% fluted Pumpkin leaf, 3% Fish) than all other samples probably because of raw materials, processing, handling, and storage. These organisms could have been introduced at the different stages of bread production. This finding is in line with the study of Daniyan and Nwokwu (2011) who identified some fungus species in bread. These organisms could be responsible for the spoilage of bread.

Table 3: Microbial load

Parameters (mg/g)	A	B	C	D
Total Plate Count (cfu/g x10^3)	3.0	1.0	1.0	2.0
E. coli (cfu/g x10^3)	NIL	NIL	NIL	NIL

Salmonella (cfu/g x10^3)	NIL	NIL	NIL	NIL
Yeast & Mould (sfu/g x10^3)	2.0	1.0	1.0	1.0

A = 86% Wheat, 11% Acha, 0% fluted Pumpkin leaf, 3% Fish, B = 77% Wheat, 12% Acha, 8% fluted Pumpkin leaf, 3% Fish, C= 70% Wheat, 17% Acha, 10% fluted Pumpkin leaf, 3% Fish, D = 100% Wheat, 0% Acha, 0% fluted Pumpkin leaf, 0% Fish

CONCLUSION

Findings from this study indicated that the incorporation of the indigenous food materials (fish, fluted pumpkin and acha) into bread production resulted in improved nutritional value by increasing mineral levels which are important in healthy bones and tooth. Consequently, it could be recommended that the use of indigenous food materials should be encouraged in the production of bread, as it is cheap and provide adequate nutrients needed.

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